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Aberdeen Proving Ground, MD 21010-5400

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Stoichiometric and catalytic scavengers as protection against nerve agent toxicity: A mini review

David E. Lenz [a,*], David Yeung [a,b], J. Richard Smith [c], Richard E. Sweeney [d], Lucille A. Lumley [a], Douglas M. Cerasoli [a]

[a] *Research Division, U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Rd., Aberdeen Proving Ground, MD 21010-5400, USA*
[b] *Department of Pharmacology and Experimental Therapeutics, University of Maryland, 655 W. Baltimore Street, Baltimore, MD 21201, USA*
[c] *Analytical Toxicology Division, U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400, USA*
[d] *RESECO Research Engineering Consultants, P.O. Box 554, Nottingham, PA 19362, USA*

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Abstract

Currently fielded treatments for nerve agent intoxication promote survival, but do not afford complete protection against either nerve agent-induced motor and cognitive deficits or neuronal pathology. The use of human plasma-derived butyrylcholinesterase (HuBuChE) to neutralize the toxic effects of nerve agents in vivo has been shown to both aid survival and protect against decreased cognitive function after nerve agent exposure. Recently, a commercially produced recombinant form of human butyrylcholinesterase (r-HuBuChE; PharmAthene Inc.) expressed in the milk of transgenic goats has become available. This material is biochemically similar to plasma-derived HuBuChE in in vitro assays. The pharmacokinetic characteristics of a polyethylene glycol coated (pegylated) form of r-HuBuChE were determined in guinea pigs; the enzyme was rapidly bioavailable with a half-life ($t_{1/2}$) and pharmacokinetic profile that resembled that of plasma-derived huBuChE. Guinea pigs were injected with 140 mg/kg (i.m.) of pegylated r-HuBuChE 18 h prior to exposure (sc) to 5.5 × LD_{50} VX or soman. VX and soman were administered in a series of three injections of 1.5 × LD_{50}, 2.0 × LD_{50}, and 2.0 × LD_{50}, respectively, with injections separated by 2 h. Pretreatment with pegylated r-HuBuChE provided 100% survival against multiple lethal doses of VX and soman. Guinea pigs displayed no signs of nerve agent toxicity following exposure. Assessments of motor activity, coordination, and acquisition of spatial memory were performed for 2 weeks following nerve agent exposure. There were no measurable decreases in motor or cognitive function during this period. In contrast, animals receiving 1.5 × LD_{50} challenges of soman or VX and treated with standard atropine, 2-PAM, and diazepam therapy showed 50 and 100% survival, respectively, but exhibited marked decrements in motor function and, in the case of GD, impaired spatial memory acquisition. The advances in this field have resulted in the decision to select both the plasma-derived and the recombinant form of BuChE for advanced development and transition to clinical trials. Efforts have now been expanded to identify a catalytic protein capable of not only binding, but also rapidly hydrolyzing the standard threat nerve agents. Recent work has focused on paraoxonase-1 (PON1), a naturally occurring human serum enzyme with the capacity to catalyze the hydrolysis of nerve agents, albeit too slowly to afford dramatic protection. Using rational design, several amino acids involved in substrate binding have been identified and site-directed mutations have revealed that residue H115 plays an important role in binding. In addition, the stereospecificity of PON1 for the catalytic hydrolysis of soman has been examined. The enzyme exhibits a slight stereospecificity for the C+P+ isomer of soman,

* Corresponding author. Tel.: +1 410 436 3525; fax: +1 410 436 8377.
E-mail address: david.lenz@us.army.mil (D.E. Lenz).

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which is due more to preferential binding than to selective hydrolysis of this isomer. The results suggest that it may be possible to engineer a mutant form of PON1 with enhanced activity and stereospecificity for the most toxic nerve agent isoforms.

Keywords: Chemical warfare nerve agents; Bioscavengers; Catalytic scavengers; Soman; Sarin; Tabun; VX

1. Background

The conventional approach to treating organophosphorus (OP) intoxication involves the use of a combination of pharmacological therapies to counteract the effects of OP-induced acetylcholinesterase (AChE) inhibition. Cholinolytic drugs such as atropine are administered to antagonize the effects of the elevated acetylcholine levels that result from the inhibition of AChE (Dacre, 1984). Additionally, an oxime such as pyridinium-2-aldoxime (pralidoxime, or 2-PAM) is given; the oxime serves as a nucleophile, reacting with the inhibited (phosphoylated) enzyme to displace the phosphyl group at the active site serine residue and restore normal AChE activity (Ballantyne and Marrs, 1992). Additionally, anticonvulsant drugs such as diazepam are also administered to control OP-induced tremors and convulsions (Maynard and Beswick, 1992). Unfortunately, these treatments can produce serious side effects if administered in the absence of cholinesterase inhibitors. For example, cholinolytics such as atropine, given in the absence of a cholinergic crisis, can cause impairment of central nervous system function due to blockade of muscarinic receptors (Grob, 1963; Somani et al., 1992). Similarly, oximes have been reported to produce transient dizziness, headache, and increases in blood pressure and heart rate (Taylor, 2001; de Candole et al., 1953; Stewart, 1959; Stewart and Anderson, 1968), as well as antinicotinic and antimuscarinic effects (Brimblecombe, 1977). Finally, in addition to their undesirable physiologic side effects, achievement of maximum effectiveness by conventional therapies requires their administration within a fairly prescribed time frame after exposure to an OP AChE inhibitor (Bajgar et al., 1971; Mesulam et al., 2002). Prompt post-exposure administration of antidotes can be facilitated by the use of auto-injectors (Bajgar et al., 1971), but this still represents a substantial logistical challenge to both civilians and soldiers in the field.

While the current therapies are effective in preventing lethality, they do not prevent performance deficits, behavioral incapacitation, loss of consciousness, or the potential for permanent brain damage (Maynard and Beswick, 1992). In an attempt to identify alternative approaches to protect against OP poisoning, efforts have focused on identifying human proteins that can remain stable in circulation for long periods of time (Dacre, 1984; Ballantyne and Marrs, 1992; Maynard and Beswick, 1992) while acting as biological scavengers for OP compounds. Biological scavenger function must be very rapid, irreversible and specific. Ideally, the scavenger should have a prolonged circulation time, be biologically innocuous in the absence of OP compounds, and not present an antigenic challenge to the immune system. Theoretically, this approach would avoid the side effects commonly associated with current therapies (de Candole et al., 1953; Stewart, 1959; Grob, 1963; Stewart and Anderson, 1968; Bajgar et al., 1971; Brimblecombe, 1977; Somani et al., 1992; Taylor, 2001; Mesulam et al., 2002). As a prophylactic, human proteins can provide protection by inactivating (through sequestration or hydrolysis) OP compounds before they can react with the target AChE. The time frame for this inactivation must occur before endogenous AChE is affected (approximately 2 min after exposure to an OP exposure in humans (Heffron and Hobbiger, 1979)). For these reasons, efforts to identify candidate bioscavengers have focused primarily on plasma-derived HuBuChE (Lenz et al., 2005; Saxena et al., 2005; Sun et al., 2005; Doctor and Saxena, 2005), although preliminary efforts with r-HuBuChE are ongoing (Cerasoli et al., 2005a).

2. Stoichiometric bioscavengers

A number of different enzymes capable of reacting with OP inhibitors but not catalyzing their hydrolysis have been tested over the past 15 years for their ability to provide protection against OP poisoning in vivo. Wolfe et al. (1987) first reported the use of exogenously administered AChE as a bioscavenger (Table 1). In that study, fetal bovine serum derived acetylcholinesterase (FBS-AChE) protected against a $2 \times LD_{50}$ dose of VX in mice (100% survival), while 80% survival was reported after a $3 \times LD_{50}$ of VX challenge. However, FBS-AChE pretreatment did not protect against soman exposure. The authors reported no detectable adverse effects as a result of administration of FBS-AChE alone.

Experiments with rhesus monkeys pretreated with FBS-AChE scavengers were also reported by Maxwell et al. (1992). In that work, monkeys pretreated with FBS-

Table 1
Protection from organophosphorus intoxication by bioscavenger

Bioscavenger	Test species	Nerve agent	Protection (LD_{50})[a]	References
eq-BuChE	Rhesus monkey	GB	1	Broomfield et al. (1991)
eq-BuChE	Rhesus monkey	GD	2 (4 w/atropine)	Broomfield et al. (1991)
eq-BuChE	Rhesus monkey	GD	5	Wolfe et al. (1992)
HuBuChE	Rhesus monkey	GD	2	Raveh et al. (1997)
HuBuChE	Rhesus monkey	VX	1.5	Raveh et al. (1997)
HuBuChE	Rat	GD	2–3	Raveh et al. (1993)
HuBuChE	Rat	VX	2	Raveh et al. (1993)
HuBuChE	Mouse	GD	2.1	Raveh et al. (1997)
HuBuChE	Mouse	GB	1.6	Raveh et al. (1997)
HuBuChE	Mouse	GA	1.8	Raveh et al. (1997)
HuBuChE	Mouse	VX	4.9	Raveh et al. (1997)
HuBuChE	Guinea pig	GD	5.5	Lenz et al. (2005)
HuBuChE	Guinea pig	VX	5.5	Lenz et al. (2005)
HuBuChE	Cynomolgus	GD	5.5	Lenz et al. (2005)
r-HuBuChE	Guinea pig	GD	5.5	Cerasoli et al. (2005a,b)
r-HuBuChE	Guinea pig	VX	5.5	Cerasoli et al. (2005a,b)
Atropine/2-PAM/DZP	Guinea pig	GD	1.5	Cerasoli et al. (2005a,b)
Atropine/2-PAM/DZP	Guinea pig	VX	1.5	Cerasoli et al. (2005a,b)

[a] Values represent multiples of median lethal doses (LD_{50}s) of nerve agent survived after bioscavenger administration.

AChE were protected with no performance decrements (assessed by serial probe recognition (SPR) task) when compared with animals treated with FBS-AChE alone or after soman challenges at both 1.5 and 2.5 × LD_{50}. Although no anti-FBS-AChE antibody response was observed, the authors cautioned that whenever a foreign protein is administered to an animal, the potential for an antibody-mediated immune response must be assessed on a case-by-case basis. Maxwell et al. (1993) also compared the relative protection against soman afforded to mice by three other treatments: pyridostigmine pretreatment with atropine therapy post-exposure, post-exposure oxime (HI-6) and atropine therapy, and FBS-AChE pretreatment alone. The authors concluded that the FBS-AChE pretreatment offered superior protection against both soman toxicity (survival after 8–10 × LD_{50} doses) and behavioral incapacitation.

Similarly, Broomfield et al. (1991) reported that equine butyrylcholinesterase (eq-BuChE) alone afforded complete protection against a 2 × LD_{50} challenge dose of soman in rhesus monkeys and against 3–4 × LD_{50} doses when atropine was administered (post-exposure). Protection against a single LD_{50} dose of sarin was also demonstrated. There were no fatalities in any of these cases. Furthermore, when animals were assessed for behavioral deficits, again using an SPR task, they all returned to baseline performance within 9 h after soman exposure (Castro et al., 1994).

In a related study, Wolfe et al. (1992) assessed the ability of pretreatment with either FBS-AChE or eq-BuChE to protect rhesus monkeys against multiple LD_{50} doses of soman. Survival and the ability to perform the primate equilibrium platform (PEP) behavioral task were the variables assessed. The animals that received FBS-AChE as a pretreatment were protected against a cumulative exposure of 5 × LD_{50} of soman and showed no decrement in the PEP task. Two of the four monkeys that received purified eq-BuChE did show some transient decrement in PEP task performance when the cumulative dose of soman exceeded 4 × LD_{50}. All of the experimental animals were observed for an additional 6 weeks, and none displayed any residual or delayed performance decrements, suggesting no residual adverse effects. Results of these and other experiments utilizing BuChE as a nerve agent scavenger are summarized in Table 1. The results of various scavenger experiments were reviewed and expanded upon by Doctor et al. (1993) in studies where mice pretreated with FBS-AChE were also administered the oxime HI-6 immediately post-exposure to sarin. In theory, the oxime will continuously regenerate the inhibited scavenger enzyme in vivo; this approach is predicted to increase the amount of sarin that could be scavenged by a given amount of AChE, endowing this stoichiometric scavenger with pseudo-catalytic properties. The therapeutic addition of HI-6 after pretreatment with FBS-AChE was found to enhance the efficacy of the scavenger enzyme against sarin in vivo, increasing the ratio of neutralized organophosphorus compound per FBS-AChE molecule from 1:1 (in the presence of AChE alone) to roughly 65:1.

The ultimate goal of scavenger molecule research is to generate a means to protect humans from the toxic effects of nerve agents. In an effort to minimize any physiological, immunological or psychological side effects of scavenger use in humans, more recent research efforts focused primarily on the use of HuBuChE, with some additional work on the model systems of human carboxylesterase (CaE) and/or FBS-AChE (which does not induce an immune response in rhesus monkeys (Maxwell et al., 1991)). In a series of studies, Ashani et al. (1991) examined the scavenger properties of FBS-AChE and particularly plasma derived HuBuChE in mice, rats and rhesus monkeys against several different nerve agents as well as other OP compounds (Raveh et al., 1993, 1997). Following administration of exogenous cholinesterase, there was a linear correlation between the concentration of cholinesterase in the blood and the level of protection against OP poisoning. Furthermore, the extent of protection afforded to mice was sufficient to counteract multiple LD_{50} doses of soman. When the protective effect of HuBuChE pretreatment was compared between mice and rats, it was found that the same linear correlation existed between blood concentration of HuBuChE and protection against soman, sarin or VX in both species (Raveh et al., 1997). They further noted that to be effective, a scavenger had to be present before exposure to the OP compound, because (as discussed above) the nerve agent must be scavenged within one blood circulation time period (Raveh et al., 1993) which is about 7 min in humans. In the final paper of this series, the authors reported similar protective results against a $3.3 \times LD_{50}$ dose of soman or a $2.1 \times LD_{50}$ dose of VX in rhesus monkeys (Raveh et al., 1997). They also report considerable protection against soman-induced behavioral deficits in a spatial discrimination task. More recently, Lenz et al. (2005) determined the pharmacokinetics and efficacy of HuBuChE in guinea pigs and cynomolgus monkeys against multiple LD_{50} challenges of nerve agents. The half-time for elimination of the plasma-derived HuBuChE was about 70–75 h for guinea pigs or cynomolgus monkeys, respectively. Guinea pigs were protected against a cumulative $5.5 \times LD_{50}$ dose of either soman or VX. At necropsy, 7 or 14 days after surviving the nerve agent challenge, all tissues appeared normal upon light microscopic examination. No signs of poisoning were observed in the experimental animals during the efficacy studies. Cynomolgus monkeys were protected against a cumulative challenge of $5.5 \times LD_{50}$ of soman as well. Of the six animals challenged, one died after the final challenge dose of soman (total dose of $5.5 \times LD_{50}$ within 4 h) and one was euthanized 48 h after the final dose of soman. The remaining animals displayed neither short-term signs of poisoning nor signs of lasting consequences as revealed by blood chemistry examinations and long-term (>20 month) observations. These efficacy results are summarized in Table 1. These studies were complimented by the work of Saxena et al. (2005), who measured the in vitro stability of plasma derived HuBuChE. It was found that the lyophilized form of the enzyme had a shelf life of over 24 months at 4, 25 or 37 °C. Additionally, reconstituted material showed no alteration in in vivo pharmacokinetic properties in either mice or rhesus monkeys. When the plasma derived HuBuChE was administered to mice or rhesus monkeys at a dose in 10-fold excess of that needed for protection against a $5 \times LD_{50}$ challenge of soman, no deficits on a variety of behavioral tasks were observed (Sun et al., 2005). Likewise, 14 days after guinea pigs were administered 60 mg/kg of HuBuChE (~10-fold higher than the assumed mg/kg human dosage for protection against $5 \times LD_{50}$s of soman), no changes were observed upon examination of histopathological, hematological, or serum chemistry parameters (Doctor and Saxena, 2005).

The supply of plasma derived HuBuChE is dependent on the availability of outdated human blood which is in turn dependent on the extent of donor participation and the fluctuating need for blood in response to natural disasters or unforeseen medical emergencies. To identify a more reliable source of HuBuChE, research efforts were focused on the development of recombinant expression systems. If successful, such efforts would enable a constant supply of material at reproducible purity and activity, thus eliminating a dependence on whole blood supply. To date, r-HuBuChE material purified from the milk of transgenic goats (Cerasoli et al., 2005b) has been the most extensively studied. In its native form, goat-milk derived r-HuBuChE is expressed primarily as a dimer, but can also be present as monomers and tetramers; plasma derived HuBuChE is predominantly tetrameric. Furthermore, goat-milk derived r-HuBuChE has a different glycosylation pattern than that of the plasma-derived material (Garcia et al., 2005). As a result goat-milk derived r-HuBuChE has a different pharmacokinetic profile, with a much shorter circulatory half-life, than plasma derived HuBuChE. To enhance its biological residence time, goat-milk derived r-HuBuChE was modified to include polyethylene glycol adducts (pegylated). The pegylated enzyme had a pharmacokinetic profile very similar to that of the plasma derived BuChE (Fig. 1) (Cerasoli et al., 2005a), suggesting that differences in pharmacokinetics between plasma purified and recombinant enzymes can be addressed using in vitro post-translational modifications (Chilukuri et

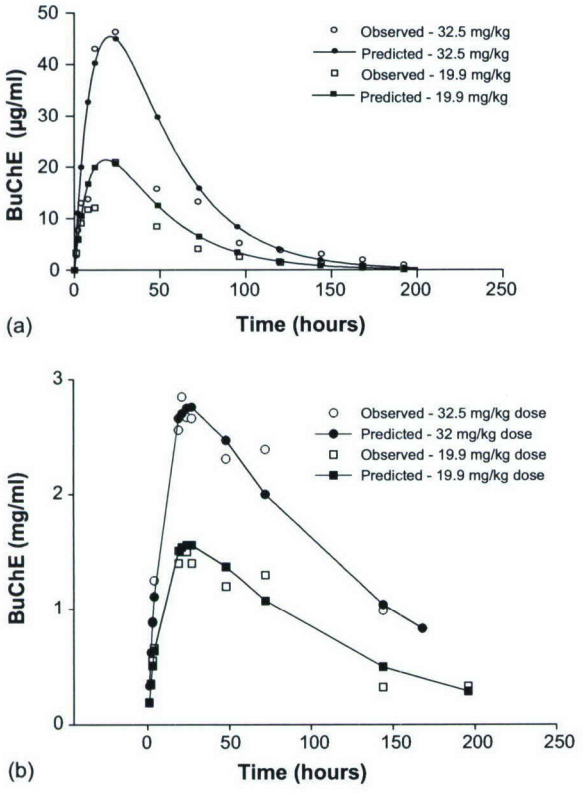

Fig. 1. (a) Time-course of HuBuChE in blood after i.m. administration in guinea pigs. (○) 32.5 mg/kg dose of HuBuChE; (□) 19.9 mg/kg dose of HuBuChE. (b) Time-course of r-huBuChE in blood after i.m. administration in guinea pigs. (○) 32.5 mg/kg dose of r-huBuChE; (□) 19.9 mg/kg dose of r-huBuChE.

al., 2005). Studies with r-HuBuChE from transgenic goat milk have yielded efficacy results in guinea pigs that are very similar to those described previously with plasma derived material, i.e., complete protection against $5.5 \times LD_{50}$ of GD or VX (Cerasoli et al., 2005a) (see Table 1). These preliminary results suggest that safe and effective recombinant stoichiometric bioscavengers can be developed, potentially providing a source for sufficient material to supply both the entire military force and possible domestic users such as first responders, emergency medical personnel, and agricultural workers that may be occupationally exposed to oxon-type OP pesticides, e.g. paraoxon, chlorpyrifos-oxon.

3. Catalytic bioscavengers

While stoichiometric scavengers afford good protection as long as they reside at high levels in circulation, they are molecules of high relative molecular weight (~86 kDa per binding site for BuChE) and a comparatively large quantity is required to neutralize an equimolar amount of nerve agent. A catalytic scavenger, even one with the same high molecular weight, administered in smaller quantities would produce the same or greater extent of protection against OP poisoning. A catalytic scavenger would also have the advantage of not being consumed in the process of detoxifying the nerve agent; it would be available to protect against multiple exposures to either high or low doses of OP inhibitors. To date, enzymes with catalytic anti-OP activity have been identified from a variety of sources, such as the OPAH from *Pseudomonas diminuta* (Serdar and Gibson, 1985), the prolidase from *Alteromonas haloplanktis* (Cheng et al., 1996), and human serum paraoxonase 1 (HuPON1) (Masson et al., 1998; Gan et al., 1991; Tuovinen et al., 1999; Josse et al., 1999, 2001). Theoretically, a functional catalytic scavenger must have both a lower K_m (inversely proportional to the strength of binding of a substrate to the enzyme) and a higher k_{cat} (turnover number) for nerve agents than have been found to date among these naturally occurring catalytic enzymes. The *P. diminuta* enzyme has been shown to afford some protection against soman lethality in mice and to protect against behavioral side effects (Broomfield, 1992). However, since this bacterially derived enzyme has no known mammalian homologues, it will likely be a potent initiator of immune responses and is therefore unlikely to be appropriate for use as a prophylactic scavenger in humans. As a result, the human serum paraoxonase 1 (HuPON1) enzyme has been identified as having a similar potential for affording protection (Table 2), but without the potential complication of inducing an immune response. It is important to note that in the case of HuPON1, the native enzyme was capable of catalytically hydrolyzing nerve agents, albeit with poor catalytic efficiency, i.e., modest affinity and a slow rate of turnover. Since OP compounds are "accidental" substrates for PON (Masson et al., 1998; Tuovinen et al., 1999), it is likely that improvement in activity can be realized through protein engineering. Unfortunately, protein engineering requires both knowledge of the three-dimensional structure of the enzyme and a detailed understanding of active site residues, which are both currently unavailable.

Recent works by Josse et al. (1999, 2001); Harel et al. (2004a,b), Aharoni et al. (2004), and Yeung et al. (2004, 2005) have partially addressed these complications regarding development of PON1 as a nerve agent bioscavenger. Josse has postulated that, based both on site directed mutations of amino acids believed to be at or near the active site of HuPON1 and on limited sequence homology with a DFPase, the molecule is a six-fold beta propeller (Fig. 2). Meanwhile, Harel et al.

Table 2
Kinetic properties of naturally occurring catalytic bioscavengers

Bioscavenger	Source species	Substrate specificity	K_m (μM)	V_{max} (nmol min^{-1} mg^{-1})	Reference
Phosphotriesterase	*P. Diminuta*	GD	36/500	15/7.3	Broomfield (1993)/Dumas et al. (1990)
Phosphotriesterase	*P. Diminuta*	GB	700	N.D.[a]	Dumas et al. (1990)
Phosphotriesterase	*P. Diminuta*	Paraoxon	50	3200	Dumas et al. (1990)
Phosphotriesterase	*P. Diminuta*	DFP	100	64	Dumas et al. (1990)

Bioscavenger	Source species	Substrate specificity	Bimolecular rate constant (k_{cat}/K_m [M^{-1} min^{-1}])	Reference
			Q191/R191[b]	
PON1	Human	GD	$2.8 \times 10^6/2.1 \times 10^6$	Masson et al. (1998)
PON1	Human	GB	$9.1 \times 10^5/6.8 \times 10^4$	Masson et al. (1998)
PON1	Human	DFP	3.7×10^4/N.D.	Masson et al. (1998)
PON1	Human	Paraoxon	$6.8 \times 10^5/2.4 \times 10^6$	Masson et al. (1998)

[a] Not determined.
[b] Two naturally occurring allelic variants of PON (Q191 and R191) have been identified. The activity of each form is shown.

(2004a) and Aharoni et al. (2004), using a bacterially expressed mouse-rat-rabbit-human chimera of PON1 obtained through gene shuffling experiments, confirmed the postulated structure through X-ray crystallographic studies. Subsequently, Yeung et al. (2004) have carried out numerous site-directed mutation studies to identify and 'map' amino acid residues critical for binding and/or catalytic activity (Table 3). More recent efforts by Yeung et al. have revealed a subtle but appreciable degree of stereospecificity in the hydrolysis of soman by

Table 3
Amino acid residues predicted to play a functional role in HuPON1 substrate binding and catalysis

Predicted role	reHuPON1	Phenyl acetate		Paraoxon	
		K_m(mM)[a]	%K_m to wt (a)	K_m(mM)[a]	%K_m to wt (a)
N-terminus signaling peptide	G11A	0.74	121	0.18	113
	G11C	0.74	121	0.19	119
	G11S	0.64	105	0.18	113
Catalytic calcium binding	N168E	nd	–	nd	–
	N224A	nd	–	nd	–
	D269E	nd	–	nd	–
Structural calcium binding	D54N	nd	–	nd	–
Substrate binding site	L69F	nd	–	nd	–
	H115W	nd	–	0.42	262
	N133S	0.59	97	0.17	106
	H115W/N133S	nd	–	0.15	83
	H134W	nd	–	nd	–
	H134Y	nd	–	nd	–
	F222D	nd	–	nd	–
	F222Y	0.89	146	nd	–
	N224A	nd	–	nd	–
	C284D	nd	–	nd	–
Catalytic site	H285D	nd	–	nd	–
	H285Y	nd	–	nd	–
Surface residue	E313A	0.51	84	0.14	88
	E314A	0.67	106	0.19	119
	V304A	nd	–	nd	–

Relative to recombinant wild-type K_m values for phenyl acetate (K_m = 0.61 mM) and paraoxon (K_m = 0.16 mM) as substrates, respectively. nd, not detectable at 3.3 and 2.6 mM substrate concentrations for phenyl acetate and paraoxon, respectively.
[a] K_m values are averages of at least three experiments with supernatants from independent transfections.

Fig. 2. Proposed model of HuPON1 showing the six-fold β-propeller structure with the two obligatory calcium atoms, one for binding and one required for stability of the molecule (Josse et al., 2002).

Table 4
Kinetic parameters for the enzymatic hydrolysis of the various GD stereoisomers by recombinant wild-type HuPON1

GD isomer	K_m (mM)	k_{cat} (min^{-1})	K_{cat}/K_m (mM^{-1} min^{-1})
C(−)P(−)	0.91 ± 0.34	501 ± 45	625 ± 241
C(−)P(+)	0.58 ± 0.23	593 ± 54	1160 ± 469
C(+)P(−)	0.71 ± 0.49	553 ± 163	1040 ± 465
C(+)P(+)	0.27 ± 0.08	1030 ± 94	4130 ± 1090

HuPON1 catalyzed GD hydrolysis was assayed in the presence of at least 1.0 mM $CaCl_2$. Kinetic results presented for each isomer were determined from at least eight independent kinetic experiments ($n = 8$).

native HuPON1, with the least toxic soman stereoisomer (C+P+) being hydrolyzed approximately six times more efficiently than the most toxic one (C-P-, Yeung et al. 2006, in press). The observed stereospecificity is due primarily to preferential binding rather than enhanced turnover of the C+P+ soman stereoisomer by HuPON1 (Table 4).

4. Summary

The threat of OP nerve agent usage not only against military personnel in the field but also against the public at large is quite real (Ember, 1991). Terrorist groups have already used nerve agents against a civilian population and due to their low cost and relative ease of synthesis, they are likely to be used again in the future (Masuda et al., 1995). Compounding the concern is the fact that many commonly used pesticides and chemical manufacturing by-products act as anticholinesterases, posing an occupational low-dose exposure hazard to workers in a variety of professions. The use of anticholinesterase pesticides against civilians in a terrorist context also exists as a possibility. Current therapeutic regimes for acute nerve agent exposure are generally effective at preventing fatalities if administered in an appropriate time frame. While the use of nerve agents on the battlefield may be somewhat predictable, their use in a terrorist situation will be, in all probability, an unanticipatable event. The potential to afford long-term protection to first responders at risk for exposure to toxic or incapacitating concentrations of OP inhibitors is a notable advantage of biological scavengers.

In addition, the use of bioscavengers has several psychological benefits that are likely to result in a higher degree of user acceptability than exists for conventional therapy. No post-exposure auto-injectors are necessary, and protection is afforded with little chance of short- or long-term side effects. Of particular significance is the fact that current candidate bioscavenger proteins are, exclusively, enzymes of human origin. From a scientific standpoint, these proteins are good candidates because they are less likely to be recognized by cells of the immune system and will enjoy prolonged residence times in circulation. From a user point of view, individuals are in essence being protected against nerve agents using a substance that their bodies already produce, rather than being injected with drugs and enzyme inhibitors that alone can produce potent side effects; such a distinction may enhance the comfort and compliance of end users.

Based on the data summarized above, the decision was made by the U.S. Army in October 2004 to transition plasma derived HuBuChE to advanced development. HuBuChE is currently being produced under good manufacturing practice (GMP) by Baxter Pharmaceuticals, using outdated human blood as the source material. This product is currently scheduled to enter Phase Ia human safety trials in late 2007, to be followed by an Investigational New Drug (IND) submission to the Food and Drug Administration (FDA). More recently (February 2006), r-HuBuChE was also transitioned to advanced development, although the details for GMP production and human clinical safety trials for this material have not yet been established. Because HuBuChE has dual use potential for both military personnel and civilian first responders, once IND status has been granted by the FDA and human safety trials are completed, this material will be

evaluated for further development to full FDA licensure.

The next phase in these efforts is to design a recombinant version of a naturally occurring human enzyme that is capable of being developed as a catalytic biological scavenger to protect against nerve agent poisoning. Since HuPON1 is a naturally occurring plasma enzyme produced in the liver, an alternative approach might be to enhance endogenous enzyme biosynthesis by inducing increased activity of the HuPON1 gene promoter. Recent results on increased expression levels of HuPON1 by HuH7 hepatoma cells upon action of fibrates are promising in this regard (Gouédard et al. unpublished).

While most of the catalytic enzymes described above have not yet been tested in mammalian systems, they are indicative of the types of therapies that may soon be available for use in animals and eventually in humans. While r-HuBuChE and mutants of HuPON1 are based on human proteins, it is recognized that the immunogenicity and serum half-life of the scavenger(s) must be determined in humans, and that efforts may be required to minimize the former and maximize the latter. Additionally, appropriate dosages of scavenger(s) must be determined that will, based on animal models, protect against concentrations of nerve agents likely to be encountered under a wide range of scenarios. While the research efforts to date have resulted in the successful transition to advanced development of stoichiometric scavengers, the use of either naturally occurring or genetically engineered enzymes with catalytic activity holds the greatest theoretical promise for the development of a broad specificity, high efficacy prophylactic scavenger. Current efforts are now focused on designing and expressing such enzymes, and characterizing their in vivo anti-nerve agent efficacy in FDA acceptable animal models.

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